DNA dynamics: **Different means to a common end?**

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A ribosomal frameshift is required for the synthesis of an essential component of the yeast telomerase pathway; this and other findings on telomerases from many species raise interesting questions regarding the evolutionary relationship between telomerases and retrotransposons lacking long terminal repeats.

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The DNA of telomeres, the protein–DNA complexes present at the ends of linear chromosomes, cannot be replicated completely by the conventional mechanisms of DNA replication. This limitation leads to loss of terminal sequences, a phenomenon termed the 'end-replication' problem. In the vast majority of eukaryotes, nuclear chromosomal DNA terminates in a region of G–T/C–A-rich simple sequences, with the G–T-rich strand proceeding in a 5′→3′ direction towards the telomere. The enzyme responsible for the synthesis of these simple telomeric sequence tracts is the ribonucleoprotein complex telomerase, first identified in ciliates [1]. This unusual enzyme uses a sequence within its RNA component as the template for the synthesis of repeats onto the termini of the G–T-rich strand.

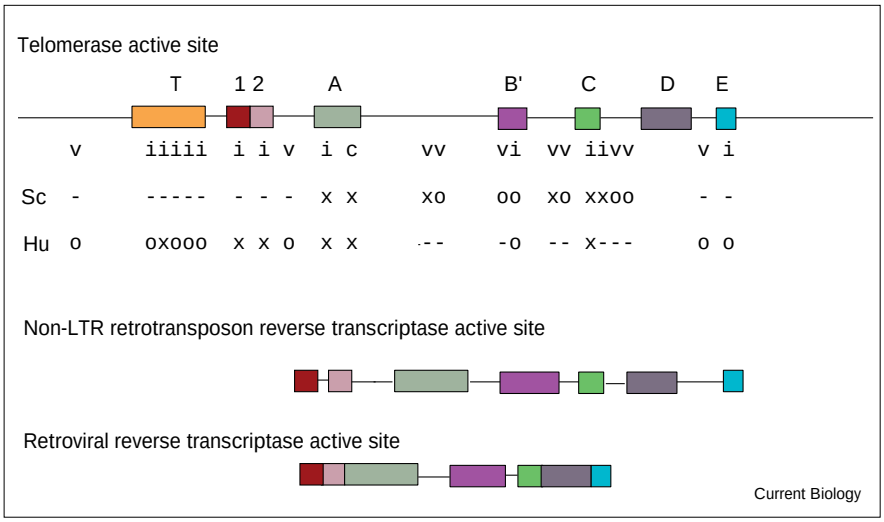
Telomerase has aroused a considerable degree of interest in recent years because of its presence in a wide variety of organisms, its novel mode of DNA synthesis and its regulation in vertebrate systems. It is of particular medical interest that proliferative cells, including germ-line and self-renewing lineages, contain telomerase activity, whereas most somatic tissues lack detectable levels of telomerase activity [2]. A high proportion of tumors contain telomerase activity, however, leading to the proposal of a relationship between telomerase activation and oncogenesis. This possibility has been strengthened by recent studies showing that the ectopic expression of telomerase prevents senescence *in vitro*, leading to a phenotype similar to cellular immortality, consistent with the oncogenesis model [3,4].

Based upon its enzymatic properties, telomerase has long been proposed to be a specialized reverse transcriptase; data supporting this claim did not emerge until very recently, however [5]. A major breakthrough was facilitated by the purification of telomerase from the hypotrichous ciliate *Euplotes*. The *Euplotes* telomerase consists of the telomerase RNA and two proteins, p123 and p43. Cloning of the gene encoding the p123 subunit revealed that this protein contained the essential motifs characteristic of reverse transcriptases (Figure 1). The identification of the *Saccharomyces cerevisiae* homolog of p123 provided the opportunity to rigorously test the role of these motifs in telomerase function [5,6].

Previous genetic studies in yeast had identified the telomerase RNA component, *TLC1*, and four proteins (Est1p–Est4p), that were essential for the maintenance of telomeric sequences [7–9]. Elimination of any one of these five factors results in yeast mutants that have identical phenotypes — a loss of telomeric sequences and a subsequent loss of viability. Epistasis analysis indicated that all five factors lie in the same genetic pathway [9]. Interestingly, one of these proteins, Est2p, is homologous to the p123 protein of *Euplotes*, particularly in the region containing the reverse transcriptase motifs [5,6]. Elegant mutational analysis of Est2p demonstrated that mutation of most residues known to be essential for the activity of other reverse transcriptases resulted in the loss of telomeric sequences *in vivo* and in the abrogation of telomerase activity (Figure 1). Est2p/p123 is therefore likely to be the catalytic subunit of telomerase, probably acting through a similar mechanism as that used by reverse transcriptases. Interestingly, Est1p, Est3p, and Est4p are not required for telomerase activity *in vitro*, even though mutation of any of these proteins causes the same phenotype of telomeric loss *in vivo* as does Est2p loss [10].

Recent studies have identified putative homologs of the Est2p/p123 protein from several organisms, including human cells [11–13]. Each of these candidates contain both a high degree of homology in the reverse transcriptase motifs and an additional telomere-specific (T) motif [11–13]: higher levels of divergence were observed outside of these domains. Several lines of evidence support the idea that the putative human counterpart of Est2p/p123 (hEst2) represents the *bona fide* telomerase catalytic subunit. First, mutations in several hEst2 residues, known to be essential for reverse transcriptase activity, eliminate telomerase activity [11–13] (Figure 1). Second, the expression pattern of *hEST2* among different tissues is tightly correlated with telomerase activity [11,12]. Third, hEst2 co-immunoprecipitates with both telomerase RNA and telomerase activity [13]. Fourth, co-expression of telomerase RNA and *hEST2* in an *in vitro* transcription–translation system generated telomerase activity [14]: this activity was eliminated by mutations in the essential conserved motifs. Fifth, expression of the

Figure 1



Mutational analysis of reverse transcriptase regions (1, 2, A, B′, C, D and E) and T motifs in telomerase. The general organization of the active-site motifs is shown relative to the organization of non-LTR retrotransposons and retroviruses [10]. A cumulative representation of mutational analysis [5,6,11,13,14] conducted in yeast (Sc) and human (Hu) cells of variant residues (v), conserved residues (c, found in majority of evolutionarily distant reverse transcriptase motifs), and invariant residues (i) within and flanking the motifs is shown. Phenotypes are denoted as follows: x, significant effect on telomerase activity and/or telomere length; o, no detectable effect on telomerase activity or telomere length; –, not tested. For the mutational analysis, the following amino acids (single-letter amino acid code) in *Saccharomyces cerevisiae* Est2p were mutated to alanine residues: D530, D536, D529, Q602, D628, Q632, D647, Q661, D670, D671, D678, and Q681. Similarly, the following amino acids in the human Est2p homolog were mutated to alanines: F487, F560, F561, Y562, T564, E565, K626, R631, R688, D712, Y717, Q833, D868, D897, and G932. The schematic diagram (not to scale) represents the relative positions of domains and mutated residues.

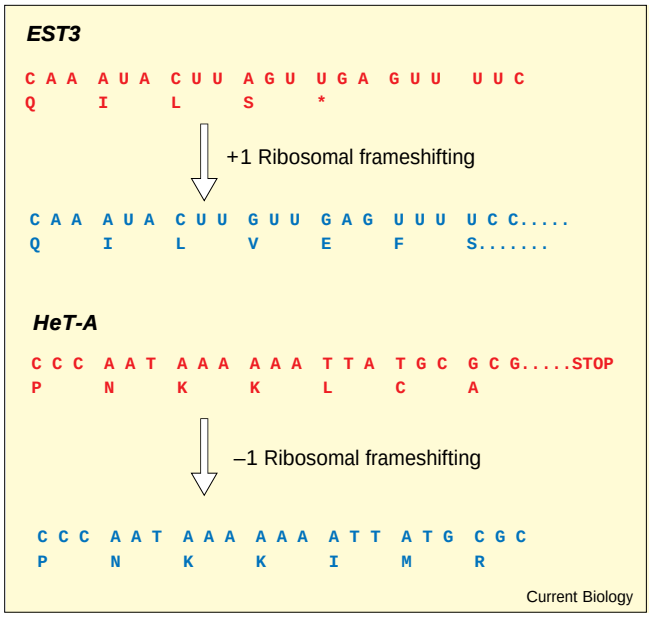
hEST2 gene in a human cell line containing the telomerase RNA but lacking telomerase activity is sufficient to generate telomerase activity in transient transfection assays *in vivo* [14]. The complete resolution of the composition of human telomerase awaits the *in vitro* reconstitution of activity from purified components, however. The emerging picture of telomerases is therefore that they contain a set of loosely conserved proteins that are most highly conserved in the region of the reverse transcriptase and telomerase-specific motifs.

Two distinct retrotransposons use reverse transcriptases conserved at the evolutionary level: long terminal repeat (LTR)-retrotransposons, and non-LTR retrotransposons, which integrate into the genome in unique ways. Several investigators have used highly-conserved regions within reverse transcriptase domains to determine the evolutionary relationship of telomerases to other reverse transcriptases [11,15]. The result depends on the manner in which the evolutionary tree is rooted. In the first case, if the evolutionary tree is rooted in ancestral RNA-dependent RNA polymerases, non-LTR retrotransposons are likely to be derived from primordial telomerases. In the second case, when the tree is rooted in prokaryotic/mitochondrial retroelements, then telomerases would be predicted to have arisen from an ancestral non-LTR retrotransposon. In either interpretation, the data point to an unmistakable link between the evolution of non-LTR retrotransposons and telomerase.

A further link has been provided by the studies of Morris and Lundblad [16] who demonstrated that an essential component of the yeast telomerase machinery, Est3p, undergoes a programmed translational frameshift, similar to that observed in some non-LTR retrotransposons. Characterization of the *EST3* gene revealed a remarkable finding — the absence of an extended open-reading frame (ORF). Only two small consecutive out-of-frame ORFs were identified (Figure 2). Morris and Lundblad noted that ribosomal frameshifting at the site of a rarely utilized codon would be expected to produce a 20 kDa protein. Consistent with this expectation, overexpression of the putative *EST3* transcript yields a 20 kDa protein as well as a smaller protein of an equivalent size to that predicted from the upstream ORF sequence. Neither protein shares any similarity with proteins currently present in the databases. Genetic analyses revealed that both the upstream and the downstream ORFs were required for the production of the full-size products and that replacement of the rare codon at the site of frameshifting with a more frequently used codon resulted in the absence of the 20 kDa product. Curiously, expression of a transcript containing in-frame versions of the two ORFs could fully complement the *est3* null mutation even though the upstream ORF was not produced. These data indicated that the 20 kDa protein, but not the product from the upstream ORF, is essential for normal *EST3* function.

Even though Morris and Lundblad [16] find no regulatory function for the Est3p frameshift, it seems unlikely that the conservation of a programmed frameshift is simply a non-functional evolutionary remnant of a shared ancestor. Indeed, the rule to date among genes encoding proteins that control telomere size is that these genes show a high rate of divergence despite their protein products serving seemingly similar roles. For example,

Figure 2



Frameshifting in the translation of yeast *EST3* and *Drosophila HeT-A* ORFs. The DNA and protein sequence surrounding the site of the frameshift are shown both before and after programmed ribosomal frameshifting. *EST3* and *HeT-A* upstream ORFs have +1 and –1 frameshifts, respectively. The stop codons for the uncorrected and translationally frameshifted *Drosophila HeT-A* ORFs are 30 amino acids and 507 amino acids downstream of the frameshift site, respectively.

although the telomerase-associated p80 proteins identified in ciliate and vertebrate cells have retained their conserved domains, these proteins are otherwise highly diverged [17–19]. Nonetheless, both of these p80 proteins clearly associate with the telomerase complex [13,17–20]. Furthermore, telomere-binding proteins from different species retain little homology in their primary sequence except for the presence of a DNA-binding domain related to that of the Myb family of transcription factors [21–23]. These proteins include the vertebrate telomere-binding protein TRF1, which has diverged rapidly even among closely related species [21].

It seems most likely, therefore, that the frameshift represents an essential function that is either retained through evolution or regained at various points during evolution. Given the lack of a detectable function for frameshifting, however, the programmed frameshift may provide an advantage over an evolutionary timescale. The presence of programmed frameshifts in retrotransposons is often used to decrease the abundance of the frameshifted product, relative to that of the upstream protein. It may well be that Est3p is a limiting factor for telomerase activity *in vivo*, preventing the promiscuous activity of telomerase which, over an evolutionary timescale or in the presence of extensive DNA damage, may have a severely deleterious effect. It will be interesting to determine whether the low abundance of Est3p limits the healing of broken ends and whether other non-catalytic components of the telomerase machinery are regulated by frameshifting.

These links between telomerase and elements of non-LTR retrotransposons are particularly interesting given that a small but diverse set of organisms, including *Drosophila* and the blue-green algae *Chlorella*, can compensate for loss of telomeric sequences through the addition of telomere-specific non-LTR retrotransposons [24,25]. The two best studied examples of these transposons are the *Drosophila HeT-A* and *TART* telomere-specific elements. Telomerase expression appears to be completely absent from *Drosophila* and maintenance of telomere size depends entirely on the frequent transposition of these two retrotransposons to chromosomal termini. The *HeT-A* element is a non-LTR retrotransposon that produces a transcript encoding a protein homologous to the retroviral Gag proteins. Interestingly, a translational frameshift is required for synthesis of the full-length HeT-A protein [26] (Figure 2). In contrast, the *TART* element contains two discrete ORFs, the first encoding a homolog of the Gag protein and the second encoding a reverse transcriptase. Non-LTR retrotransposons appear to use the 3′ hydroxyl group of a DNA end as a primer for reverse transcription, making them well suited for telomere-specific transposition [27].

Although an evolutionary link between non-LTR retrotransposons and telomerases seems unmistakable, the data should be interpreted more cautiously when attempting to correlate the evolutionary history of telomere-specific retrotransposons with that of telomerases. The complication of such an approach is the inherent assumption that only a single means of telomere addition is present in any organism. A large body of data indicates otherwise, however, and suggests that multiple means of protecting the termini from degradation might have co-evolved. For example, in the yeast *S. cerevisiae*, a ‘secondary’ pathway involving recombination can overcome the requirement for telomerase, allowing continued growth of the population [28]. In addition, recent studies have identified a set of LTR-transposable elements, *Ty5*, that preferentially insert into regions adjacent to telomeres, providing a third potential system for protection of the terminus [29].

Similar secondary recombinational pathways have been found in cells from a multiplicity of organisms, including humans [30]. Indeed, at least some members of the *Lilium* family seem to utilize recombination between LTRs as the primary mechanism for controlling telomere size [31]. This idea of multiple pathways would also be most consistent with the finding that the structurally distinct *HeT-A* and *TART* elements have a similar end-protection mechanism. Of particular relevance, however, is the recent

observation that *Chlorella* telomeres seem to have two active means of telomere maintenance: telomerase-mediated replication and non-LTR retrotransposition [25]. Some chromosome ends in *Chlorella* contain canonical telomeric repeats, with the *Zepp* retrotransposon present in multiple copies in subtelomeric locations: however, in at least one telomere, simple-sequence repeats appear to have been lost altogether, leaving a Zepp retrotransposon at the extreme terminus.

The co-evolution of alternative mechanisms for telomere maintenance would predict that elimination of the telomerase pathway would simply uncover a second pathway. Rather than revealing an evolutionary relationship, the presence of a limited set of 'back-up' mechanisms may instead be an indicator of the limited mechanisms — telomerase-mediated replication, transposition, and recombination — that can be used by the cell for end protection. In some cases, these secondary processes may have evolved later than telomerase and possibly independently of this ribonucleoprotein complex. The puzzle of the evolutionary interrelationships among mechanisms for solving the end-replication problem will provide a unique challenge for the future.

References

1. Greider CW, Blackburn EH: Identification of a specific telomere terminal transferase activity in ***Tetrahymena*** extracts. *Cell* 1985, 43:405-413.
2. de Lange T: Telomere dynamics and genome instability in human cancer. In *Telomeres*. Edited by Blackburn EH, Greider CW. Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 1995:265-293.
3. Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu C-P, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE: Extension of life-span by introduction of telomerase into normal human cells. *Science* 1998, 279:349-352.
4. Vaziri H, Benchimol S: Reconstitution of telomerase activity in normal human cells leads to elongation of telomeres and extended replicative life span. *Curr Biol* 1998, 8:279-282.
5. Lingner J, Hughes TR, Shevchenko A, Mann M, Lundblad V, Cech TR: Reverse transcriptase motifs in the catalytic subunit of telomerase. *Science* 1997, 276:561-567.
6. Counter CM, Meyerson M, Eaton EN, Weinberg RA: The catalytic subunit of yeast telomerase. *Proc Natl Acad Sci USA* 1997, 94:9202-9207.
7. Lundblad V, Szostak JW: A mutant with a defect in telomere elongation leads to senescence in yeast. *Cell* 1989, 73:347-360.
8. Singer MS, Gottschling DE: ***TLC1***, the template RNA component of the ***Saccharomyces cerevisiae*** telomerase. *Science* 1994, 266:404-409.
9. Lendvay TS, Morris DK, Sah J, Balasurbramanian B, Lundblad V: Senescence mutants of ***Saccharomyces cerevisiae*** with a defect in telomere replication fidelity identify three additional ***EST*** genes. *Genetics* 1996, 144:1399-1412.
10. Lingner J, Cech TR, Hughes TR, Lundlbad V: Three ever shorter telomere (EST) genes are dispensable for ***in vitro*** telomerase activity. *Proc Natl Acad Sci USA* 1997, 94:11190-11195.
11. Nakamura TM, Morin G, Chapman KB, Weinrich SL, Andrews WH, Lingner J, Harley CB, Cech TR: Telomerase catalytic subunit homologs from fission yeast and human. *Science* 1997, 277:957-959.
12. Meyerson M, Counter CM, Eaton NG, Ellisen LW, Steiner P, Caddle SD, Ziaugra L, Beijerbergen RL, Davidoff MJ, Liu Q, *et al.*: ***hEST2***, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. *Cell* 1997, 90:785-795.
13. Harrington L, Zhou W, McPhail T, Oulton R, Yeung DSK, Mar V, Bass MB, Robinson MO: Human telomerase contains evolutionary conserved catalytic and structural subunits. *Genes Dev* 1997, 11:3109-3115.
14. Weinrich SL, Pruzan R, Ma L, Ouellette M, Tesmer VM, Holt SE, Bodnar AG, Lichtsteiner S, Kim NW, Trager JB, *et al.*: Reconstitution of human telomerase with the template RNA component hTR and the catalytic subunit hTRT. *Nat Genet* 1997, 17:498-502.
15. Eickbush TR: Telomerase and retrotransposons: which came first? *Science* 1997, 277:911-912.
16. Morris DK, Lundblad V: Programmed translational frameshifting in a gene required for yeast telomere replication. *Curr Biol* 1997, 7:969-976.
17. Collins K, Kobayahsi R, Greider CW: Purification of ***Tetrahymena*** telomerase and cloning of genes encoding the two protein components of the enzyme. *Cell* 1995, 81:677-686.
18. Harrington L, McPhail T, Mar V, Zhou W, Oulton R, Amgen EST Program, Bass MB, Arruda I, Robinson MO: A mammalian telomerase-associated protein. *Science* 1997, 275:973-979.
19. Nakayama J-I, Saito M, Nakamura H, Matsuura A, Shikawa F: ***TLP1***: a gene encoding a protein component of mammalian telomerase is a novel member of WD repeats family. *Cell* 1988, 88:875-884.
20. Ramakrishman S, Sharma HW, Farris AD, Kaufman KM, Harley JB, Collins K, Pruijn GJM, van Venrooij WJ, Martin ML, Narayanan R: Characterization of human telomerase complex. *Proc Natl Acad Sci USA* 1997 94:10075-10079.
21. Konig P, Giraldo R, Chapman L, Rhodes D: The crystal structure of the DNA-binding domain of yeast RAP1 in complex with telomeric DNA. *Cell* 1996, 85:125-136.
22. Bilaud T, Koering CE, Binet-Brasselet E, Ancelin K, Pollice A, Gasser SM, Gilson E: The telobox, a Myb-related telomeric DNA binding motif found in proteins from yeast, plants and human. *Nucleic Acids Res* 1996, 24:1294-1303.
23. Smith S, de Lange T: TRF1, a mammalian telomeric protein. *Trends Genet* 1997, 13:21-26.
24. Pardue ML, Danilevskaya ON, Lowenhaupt K, Slot F, Traverse KL: Drosophila telomeres: new views on chromosome evolution. *Trends Genet* 1996, 12:48-52.
25. Higashiyama T, Noutoshi Y, Fujie M, Yamada T: Zepp: a LINE-like retrotransposon accumulated in the ***Chlorella*** telomeric region. *EMBO J* 1997, 16:3715-3723.
26. Biessmann H, Kasravi B, Bui T, Fujiwara G, Champion LE, Mason JM: Comparison of two active HeT-A retroposons of ***Drosophila melanogaster***. *Chromosoma* 1994, 103:90-98.
27. Finnegan, DJ: Transposable elements: How non-LTR retrotransposons do it. *Curr Biol* 1997, 7:R245-R248.
28. Lundblad V, Blackburn EH: An alternative pathway for yeast telomere maintenance rescues ***est1***$^{-}$ senescence. *Cell* 1993, 73:347-360.
29. Zou S, Voytas DF: Silent chromatin determines target preference of the ***Saccharomyces*** retrotransposon ***Ty5***. *Proc Natl Acad Sci USA* 1997, 94:5412-5416.
30. Bryan TM, Marusic L, Bacchetti S, Namba M, Reddel RR: The telomere lengthening mechanism in telomerase-negative immortal human cells does not involve the telomerase RNA subunit. *Hum Mol Genet* 1997, 6:921-926.
31. Pich U, Fuchs J, Schubert I: How do ***Alliaceae*** stabilize their chromosome ends in the absence of TTTAGGG sequences? *Chromosome Res* 1996, 4:207-213.